CHAPTER 18

Control of Bacterial Growth in Stored Maple Sap by Irradiation of the Sap Surface with Germicidal Lamps*

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Growth of adventitious bacteria in stored maple sap causes deterioration which results in the production of low grade maple sirup. This study was made to determine the effectiveness of commercial germicidal lamps, used under different conditions, for the destruction or control of bacterial populations in stored sap. The lamps were so mounted above the sap as to irradiate its entire surface. Irradiation of static sap was found unsuitable, since more than 50% of the ultraviolet radiation was absorbed in the first inch of sap and its effect on bacteria below that depth was negligible. Continuously renewing the sap surface layer by recirculation effectively controlled or decreased the bacterial population. The study shows that the effectiveness of the germicidal lamp was determined by the rate of surface renewal. The effect of the temperature of the sap on the extent of control obtained with the germicidal lamp was also studied.

It has long been recognized that the sanitary quality of maple sap is a controlling factor in the manufacture of maple sirup. Maple sap, as it leaves the vessels of the tree, is sterile; but it is readily contaminated by microorganisms introduced during handling and storage prior to the atmospheric boiling process, which cause it to be degraded. Maple sirup made from fermented sap is dark in color and may have poor flavor and in some instances poor texture (ropiness). The sap producer and evaporator operator have recognized that sap contamination and subsequent microbial fermentation must be controlled to minimize sap spoilage. Without this control, sap cannot be held more than 24 hr before the effects of fermentation are noticed. Downgrading of the sirup results, causing severe economic loss to the sap producer. The control or prevention of bacterial growth and its resulting fermentation products (principally reducing sugars) in raw sap during storage prior to the sterilizing effect of evaporation is therefore of major importance.

Conventional chemical germicides and bacteriostatic compounds cannot be used because of their adverse effects on the flavor and color of the sirup. Moreover, the evaporation process concentrates the sap approximately fortyfold. This would increase the concentration of an additive to an unsafe and nonpermissible level. Physical methods of preventing or controlling microbial growth in sap provide an excellent alternative to the use of additives. Of these, ultraviolet irradiation is best suited for sap preservation. The actinic rays of ultraviolet light have no effect on the flavor or color of sirup made from irradiated sap. Irradiation poses no such problem as results from germicidal additives; and the cost of equipment and power is nominal. Previous studies showed that solar ultraviolet irradiation of maple sap contained in transparent

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plastic bags controlled bacterial growth (Naghski and Willits, 1953). Laboratory studies showed that ultraviolet lights emitting actinic rays in the range of 260-270 mμ were effective in the control of bacterial contaminants in sap (Frank and Willits, 1960; Schneider et al., 1960). These studies indicated that microbial contaminants of sap could be controlled effectively with ultraviolet lights. In-line ultraviolet irradiation units could be used to decrease the bacterial population of maple sap by more than 95%. However, without further control, the surviving cells multiplied and deteriorated the sap during prolonged storage, especially if the sap was warm (Kissinger and Willits, 1966a).

During the 1964 and 1965 sap seasons, limited studies were conducted on the laboratory grounds in which the sap was stored in roadside tanks with the sap surface continually irradiated by overhead ultraviolet lamps (Kissinger and Willits, 1966b). This sap, collected and transported in transparent plastic tubing, had very low bacterial counts when it was delivered to the tanks. Even though depths of sap stored in the tanks reached 28 inches, the bacterial counts in the sap were maintained below 4.0×10^5 organisms/ml and, after 11 days' storage, a top grade, light amber sirup was produced from this sap. In a comparable sap without the overhead irradiation, uncontrolled growth by adventitious microorganisms during a 3 days' storage caused production of sirup at least two grades darker. It was further observed that sap obtained late in the sap flow season, when the air and sap temperatures were high (65 F), and the depth of the accumulated sap in the tank was as much as 28 inches, the bacterial count of the sap increased from an initial 1.2×10^3 organisms/ml to 4×10^5 in 5 days. Temperature, depth of sap, and possibly other factors influence the effectiveness of ultraviolet irradiation in controlling microbial growth in sap. No data are available showing the effective penetration of the actinic rays of ultraviolet light or their effects on bacterial growth at different depths in maple sap. Studies were therefore conducted to determine this and to develop means for controlling microbial growth in stored sap.

Experimental

Apparatus

1) A 56-gal galvanized iron sap storage tank, 34 inches long × 8¾ inches wide × 43½ inches deep is shown in Figure 1. The tank was securely braced on all sides to prevent deformation from hydrostatic pressure. Sampling ports were spaced at 6-inch intervals from top to bottom along the midline of the long side. The sample ports were holes cut in the tank. They were fitted with Arthur H. Thomas* No. 10 stoppers with recessed tops. The stoppers were shortened at the solid end so that the thickness of rubber was ¼-inch, permitting penetration of a hypodermic needle for sampling. A thermometer (0-200 F) was mounted in the thermometer well, and an electronic temperature controller with a sensor was mounted on the thermometer to regulate the heat supplied by the Calrod heating coil.

2) A tank identical to tank No. 1 except that it was provided with a circulation system is shown in Figure 2. The circulation system consisted of two ⅝-inch O.D. copper tubes, each 34 inches long. Both tubes were provided with ⅛-inch orifices spaced at 3-inch intervals along their entire length. One tube, mounted ½-inch above

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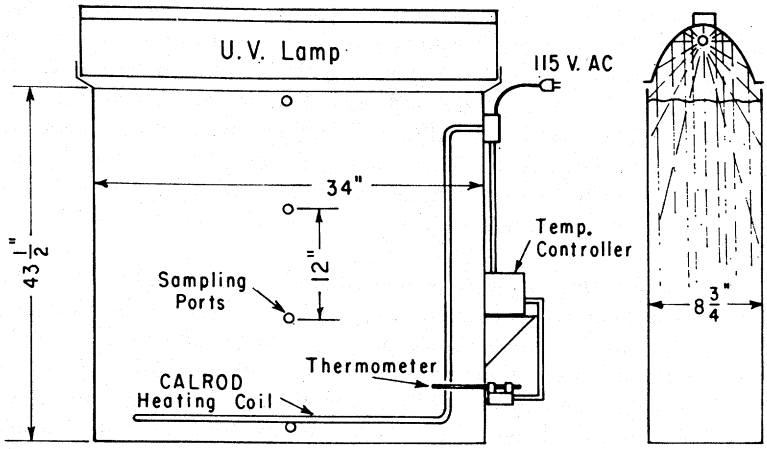

FIG. 1. Storage tank and ultraviolet lamp assembly for statically stored sap.

the bottom of the tank, served as the suction line, and the other, mounted 1½ inches below the rim of the tank, was the discharge line. The suction line was connected to a centrifugal pump which, in turn, was connected consecutively to a needle valve and to a flow meter (graduated in 0.1 gal/min), and finally to the discharge line.

3) Germicidal lamps. A 30-watt (32-inch effective length), General Electric* No. G30T8 ultraviolet lamp with an average output of 6.6 watts and with maximum intensity of 1.13 watts/ft² at 6 inches from, and perpendicular to, the bare tube.

4) Germicidal lamp fixture. A Sim-Kar No. 20086 lamp fixture, 36 inches long with an SRW/30 symmetrical reflector, was mounted above each tank so that the ultraviolet tubes in the fixtures were 6 inches above and centered over the sap surface.

5) Calrod heater. A 115-volt flexible immersion heater (Aminco Lolag*) was mounted in each tank.

6) Temperature controller. A Thermowatch* electronic temperature controller controlled operation of the Calrod heater.

7) Synthetic sap. Because whole fresh sap was unavailable at the time these studies were made, all of the studies were carried out with a synthetic sap made by dilution of light amber maple sirup to 2.5° Brix with sterile water, adding 0.9 g/gal of sugar sand (the organic acid salts precipitated when the sap is concentrated to sirup) to the dilution. The very light amber (fancy grade) sirup was used to minimize absorbance of ultraviolet rays by color in the synthetic sap.

8) Sampling syringes. Samples were taken from the sampling ports with 5.0-ml B. D. Luer-Lok syringes fitted with 20-gage, 2-inch hypodermic needles.

9) Bacterial inoculum. A mixed culture containing *Pseudomonas, Bacillus, Leuconostoc,* and other genera that are normal to maple sap was obtained from commercially produced sap. The mixed culture was maintained on tryptone glucose extract agar (Difco*) slants at 30 C and kept viable by transferring at 48-hr intervals. The inoculum was started by making a suspension of the mixed organisms from a 48-hr slant culture in 10 ml of sterile synthetic sap. The suspension was used to inoculate

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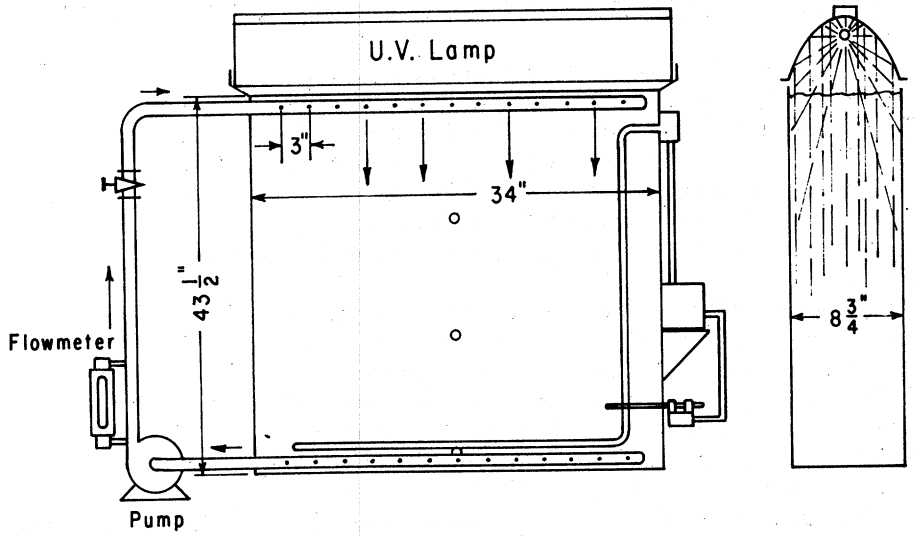

FIG. 2. Storage tank for recycling sap and ultraviolet lamp assembly.

150 ml of sterile synthetic sap in a Roux flask. It was incubated for 48 hr at 30 C, and used to inoculate 1 gal of sterile synthetic sap which was, in turn, incubated at 30 C for 48 hr. The cell concentration of the latter was determined using a hemacytometer. Based on this count, an amount of the final inoculum was added to the synthetic sap in the irradiation tank sufficient to yield a cell concentration of approximately 1×10^6/ml.

Procedure

1) All of the irradiation studies were conducted with the tanks of stored sap located in a 35 F constant temperature room.

Studies on statically stored sap. The germicidal effects of ultraviolet irradiation on bacteria in statically stored sap were measured with the sap stored at 35 F and 55 F respectively. Before each study, the tank (Fig. 1) was sanitized with 0.5% alkaline hypochlorite solution, followed by three successive rinses with tap water. For the study at 35 F, the sanitized tank was filled with synthetic sap and allowed to stand until the temperature of the sap was stabilized at the temperature of the constant temperature room, 35 F. The mixed culture inoculum was added and dispersed through the sap by stirring, and the inoculated sap was allowed to stand for 2 hr to restore the static state. Samples for bacterial counts were then taken simultaneously at (a) ¼-inch below the center of the surface area and (b) from the sampling ports at depths of 1, 2, and 3 ft. The times at which these samples were taken was noted as zero irradiation time. Immediately after this sampling, the ultraviolet lamp was turned on. The lamp assembly and upper part of the tank was covered with a hood of aluminum foil to exclude air contaminants and to shield workers in the area against the effects of the ultraviolet radiation. Sampling was repeated at 24-hr intervals for 4 successive days. For the studies made on sap stored statically at 55 F, the tank was filled with synthetic sap and heated to 55 F with the Calrod heater (Fig. 1). The temperature was held constant by the electronic controller.

Inoculation and sampling followed the same procedures used for the 35 F storage study.

Studies on recirculated sap. The tank equipped with the recirculating system (Fig. 2) was sanitized by filling the tank with a 0.5% alkaline hypochlorite solution. This was pumped through the recirculating system for 1 hour at the rate of 1 gal/min. The sanitization was followed by draining the tank completely and rinsing the system with tap water which was pumped through the system for 5 min. The tank was drained completely and filled with the synthetic sap, brought to the desired temperature by means of the electronic temperature controlled heater and the recirculating system.

The inoculum which had been prepared as before was then added and dispersed by stirring, followed by recirculation at 1 gal/min for 10 min. The desired flow rate was then set and the zero irradiation time samples for bacterial counts taken from ¼-inch below the surface and from the sample port at the 3-ft depth. The ultraviolet lamp was then turned on and the sampling process was repeated at 1-hr intervals over a period of 6 hr. The procedure was repeated for each temperature and recirculation rate studied.

2) Sampling. Samples for bacterial counts were taken aseptically from ¼-inch below the sap surface with sterile, 5-ml pipets. Samples were taken aseptically from the sample ports using the sterile 5-ml syringes fitted with 20-gage, 2-inch long hypodermic needles. The sample ports were sanitized by rinsing with a 0.5% alkaline hypochlorite solution and the 2-inch long hypodermic needles were inserted full-length into the recessed area of the rubber stopper closures in the sampling ports to withdraw 5-ml samples.

3) Plating. Tryptone glucose extract agar (Difco) was used as the plating medium for bacterial counts. All plates were incubated at 30 C for 48 hr, and counts were made with a Quebec colony counter.

Results and Discussion

The lethal effect of the ultraviolet irradiation supplied by the overhead lamp on the bacterial population of statically stored maple sap at 35 F is shown in Figure 3. The actinic rays of the lamp were highly effective in killing organisms in the top ¼-inch (surface) layer of the stored sap. The bacterial population was reduced from the initial count of 1.0×10^6 cells/ml to less than 1/ml during the first 24 hr. The bacterial population at the 1, 2, and 3-ft depths was reduced 99%, 92%, and 75%, respectively, at the end of 24 hr and was still at the 91% level at the 2-ft depth after 72 hr. The samples taken at the 3-ft depth first showed a decrease in bacterial population from the initial count of 1×10^6 cells/ml to 3.5×10^5 at the end of 72 hr, then a slight increase in population to 5.4×10^5 cells/ml at the end of 96 hr. Figure 3 shows that the penetration of the lethal actinic rays of ultraviolet light diminished with increased depth of sap. The slight decrease in bacterial count at the 3-ft depth indicates that only a limited amount of the actinic rays penetrated to this depth. Since the population at the 3-ft depth shows a slight but normal growth rate after 72 hr at this temperature, it is likely that the early small decrease in bacterial population at this depth may be due to the combined effect of the actinic rays and of environmental change (temperature) in the growth medium. The germicidal action of the ultraviolet radiation is almost complete at the surface of the statically stored sap, but diminished rapidly with depth and was negligible at the 3-ft level.

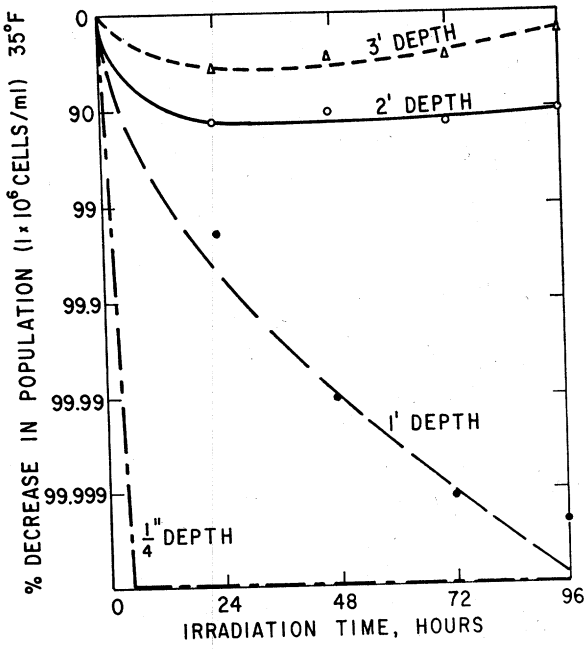

FIG. 3. Decrease in a bacterial population of 1×10^6 cells/ml at different depths in sap stored statically at 35 F and irradiated by an overhead ultraviolet lamp.

Although commercially stored sap seldom reaches a temperature of 55 F, a study was conducted with sap held at this temperature to see whether the higher temperature carried an increased lethal effect of the ultraviolet radiation. It has been reported that irradiation effects increase sharply as the temperature increases from 30 to 70 F (Cortelyou, et al., 1954, and General Electric Company, 1965). The study with sap held statically at 55 F was also made to observe the interaction of the two effects: the enhanced lethal effect of the actinic rays of the ultraviolet lamp, and the counter effect of increased bacterial growth rate resulting from the higher storage temperature. The results of this study are shown in Figure 4. As in the previous study, the samples taken ¼-inch below the surface had bacterial counts of less than 1 cell/ml for the different exposure times. During the first 24 hr of irradiation, the bacterial population decreased by 94%, 70%, and 15% at the 1-ft, 2-ft, and 3-ft depths, respectively. Then a steady and measurable increase in counts took place until after 96 hr irradiation, the original bacterial count was reached at the 2-ft level and was exceeded at the 3-ft depth. Thus, the increase in irradiation effect derived from increasing the sap temperature was counteracted by the more rapid bacterial growth at the higher storage temperature (Ingraham and Stokes, 1959).

The successful storage of maple sap requires that no microbial degradation of the sap occurs. The initial bacterial population of the stored sap must be reduced to a minimum number in the shortest possible time and kept at that level. Figures 3 and 4 show that ultraviolet irradiation of statically stored sap fails to do this unless the temperature of the sap is low and the depth of the irradiated sap is less than 2 ft and preferably not more than 1 ft. Both of these conditions would be difficult to meet in commercial operations.

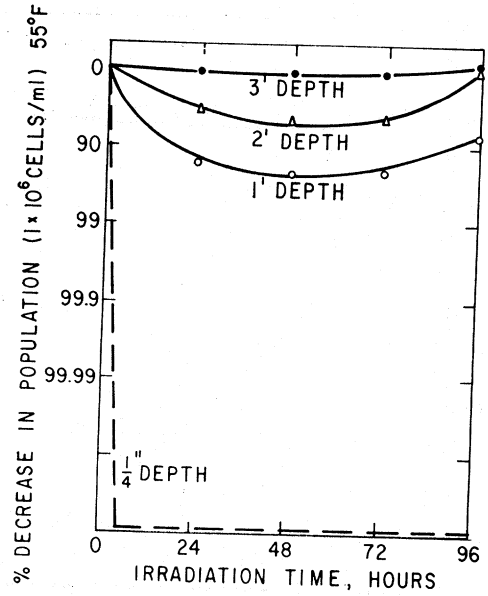

FIG. 4. Decrease in a bacterial population of 1 × 16^6 cells/ml at different depths in sap stored statically at 55 F and irradiated by an overhead ultraviolet lamp.

The previous studies demonstrated the effectiveness of the ultraviolet irradiation in reducing bacterial populations at the surface of stored sap. This suggested that better control of bacterial contaminants in stored sap, regardless of its temperature or depth, could be developed by continuously renewing the surface of the sap exposed to the actinic rays. This could be done by inducing currents in the sap that produce a rolling rather than a swirling motion with a recirculating pump.

The tank used in the static storage studies was modified so that the sap surface could be continuously renewed by means of the recirculating pumping system shown in Figure 2. This system of surface renewal was used rather than one utilizing an agitator because it permitted reproducible recirculating conditions. It was designed so that the sap would be removed from the bottom of the tank along the entire long dimension of the tank and returned to the tank at a point just below the sap surface along the entire long dimension. Thus, each time a volume of sap equal to the volume of the tank was recirculated it was assumed that in that time period all of the sap in the tank had passed through the surface layer of the sap. Thus, at a recirculation rate of 0.5 gal/min all of the 60 gal of sap in the storage system (56 gal in the tank and 4 gal in the pump, flowmeter, and piping) had passed through the surface strata of the stored sap in 2 hr.

A study was conducted in which the surface of sap stored at 55 F was continuously renewed by recycling at pump rates of 0.1, 0.25, 0.5, and 0.75 gal/min, giving recycle times corresponding to 10 hr, 4 hr, 2 hr, and 1⅓ hr, respectively. The results of this study showing the changes in bacterial populations at the 3-ft level with different rates of renewal of the sap surface exposed to ultraviolet irradiation are given in Fig. 5. All samples taken at the sap surface (¼-inch depth) in the course of this study had bacterial counts of less than 1 organism/ml and are not shown in Figure 5. As expected, the greatest decrease in bacterial population at the 3-ft level occurred at the most rapid

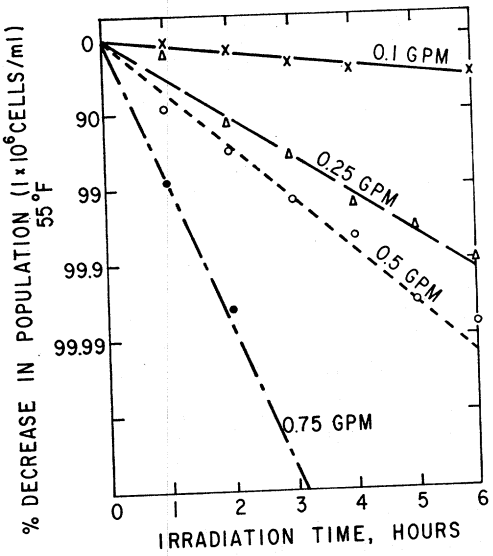

FIG. 5. Decrease in a bacterial population of 1×10^6 cells/ml in sap stored at 55 F and recirculated at different recycle rates under ultraviolet irradiation.

recirculation rate, with a 99% decrease occurring during the first hour of irradiation. Even at the much slower recirculation rate of 4 hr (pump rate of 0.25 gal/min), the population was reduced more than 90% after only 2 hr irradiation. The logarithm of the decrease in bacterial population plotted against the different rates of surface renewal produced a straight line.

The study was repeated with sap stored at 35 F to observe the effect of lower temperature on the survival of bacteria in sap recirculated under ultraviolet irradiation. The results of this study are shown in Figure 6. As in the previous study, all samples taken at the ¼-inch depth, regardless of time of irradiation contained less than 1 organism/ml, and are not plotted. At the 3-ft depth, the most rapid decrease in the bacterial population in the sap occurred at the highest recirculation rate. However, much longer times of irradiation were required to cause comparable population decreases at all recirculation rates to those observed for sap stored at 55 F.

Because of this apparent reversal in population decrease in the recycled, irradiated sap as compared to the sap stored statically at the same temperature, the temperature effect over a wide range of temperatures was studied. The recycling time was kept constant (pump rate of 0.5 gal/min) with sap stored at 35, 45, 55, and 65 F. The results are shown in Figure 7. Samples taken at the surface (¼-inch depth) contained less than 1 organism/ml. As in the previous studies, a uniform decrease in bacterial population was noted at each temperature at the 3-ft depth as time of exposure to the ultraviolet irradiation increased. A straight line was produced when decrease in population was plotted against time of exposure on semi-log paper. The greatest decrease in population is shown at the highest temperature (65 F) and the smallest at the lowest temperature (35 F), confirming the temperature effect described earlier. Bacteria in the surface layer of sap were killed very rapidly. Under the conditions used in this study, the germicidal activity of the ultraviolet irradiation took place irrespective of temperature. The growth rate of the bacteria had little effect on the

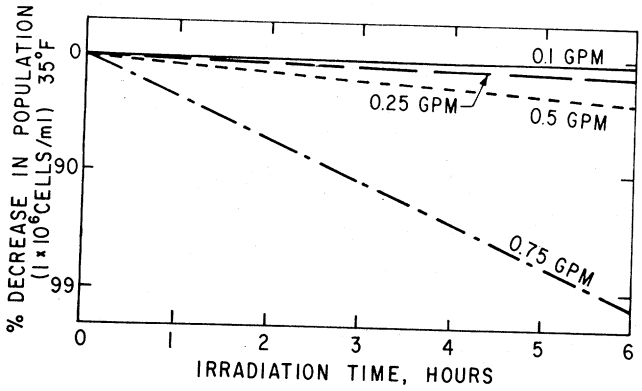

FIG. 6. Decrease in a bacterial population of 1×10^6 cells/ml in stored maple sap recirculated at different recycle rates under ultraviolet irradiation at 35 F.

results because at the temperatures used the regeneration times were very long. Unpublished data from previous studies at this laboratory showed that even at 65 F, the highest temperature used, more than 1¼ hr was required for the bacterial population to double. On the other hand, since the ultraviolet lamp and reflector were covered, the lamp was approximately at the same temperature as the stored sap, and it has been reported that a decrease in temperature causes a pronounced decrease in output of lamp energy (General Electric Co., 1965). Hence, the decrease in germicidal activity observed at the lower temperatures shown in Figure 7 could be attributed mainly to the decrease in output of lamp energy.

The results shown in Figures 5, 6, and 7 indicate that much better control of bacterial populations in stored maple sap was achieved by recycling the sap under ultraviolet irradiation than by irradiation of statically stored sap. Even at the lowest recycle rate (0.1 gal/min), decreases in bacterial population were obtained at the 3-ft depth during the 6-hr recirculation period at all storage temperatures. When more rapid recycle rates and storage temperatures above 35 F were used, bacterial counts at the 3-ft. depth in the sap were reduced by more than 95% during the 6-hr recirculation period. Static storage of sap under ultraviolet irradiation did not result in a comparable reduction in bacterial population at the 3-ft sap depth, and at 55 F the bacterial count of the sap increased at the 3-ft depth during the 96-hr storage period. The control or inhibition of bacterial growth in stored sap which is a prerequisite for the production of high quality maple sirup can be achieved by continually renewing the sap surface by recirculation under ultraviolet irradiation.

This study will be followed by field studies on commercially stored sap where sap will be irradiated by ultraviolet light under different conditions.

SUMMARY

1) A method has been developed for controlling or reducing the bacterial population of stored maple sap.

2) The method utilizes ultraviolet irradiation of the stored sap by 30-watt (36-inch long) ultraviolet lamps emitting radiation in the region of 260-270 mμ

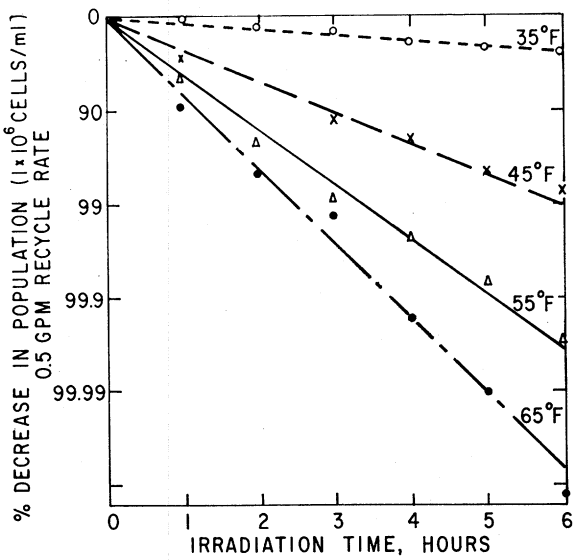

FIG. 7. Decrease in a bacterial population of 1×10^6 cells/ml in stored maple sap recycled at 0.5 gal/min under ultraviolet irradiation at different storage temperatures.

3) The bacterial population can be controlled in sap stored statically at 35 F by irradiation with germicidal lamps. At the 3-ft depth the bacterial population is decreased by 75% in 24 hr and to 65% in 72 hr. Progressively faster control is obtained with decreasing depths.
4) Ultraviolet irradiaton of sap stored statically at 55 F prevented any increase in bacterial population at the 3-ft depth during 72 hr of storage and caused decreases in bacterial population at the 1 and 2 ft depths.
5) Continuously renewing the surface of sap stored under ultraviolet irradiation by use of a recirculating pump gives complete control of the bacterial population of the sap and is independent of the sap depth and temperature.
6) The effectiveness of ultraviolet irradiation in controlling bacteria in recirculating stored maple sap increases as the rate of surface renewal increases.

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